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GENETIC VARIABILITY IN SOME NEW ZEALAND-GROWN VARIETIES OF BARLEY AND RYE

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ABSTRACT

Gel electrophoresis is used to measure allele and genotypic frequencies at esterase and acid phosphatase loci in local cultivars of barley (*Hordeum distichum*) and rye (*Secale cereale*). These frequencies are used to compute percentage heterozygosity (barley 4.27%, rye 27.55%) and a polymorphic index (barley 0.135, rye 0.216) by which the genetic variability of the two species is compared. Although low, the variability of the barley cultivars is significant. Analysis of the possible forces responsible for this variability indicates that balancing selection plays the predominant role in opposing the unifying force of inbreeding. Measurement of selective values shows the heterozygotes are favoured over twice as much as the homozygotes.

INTRODUCTION

The importance of the degree of inbreeding in determining the genetical structure and evolutionary potential of a population has long been discussed. Outbreeders are said to maintain genetic variability as heterozygotes by such mechanisms as balanced polymorphisms, coadaption and genetic homoeostasis (Dobzhansky 1955). By contrast, inbreeding populations are thought to store genetic variability in diverse arrays of sympatric homozygous lines which are occasionally capable of intercrossing and so eventually generating a new set of homozygous lines (Stebbins 1950, 1957). However, it had been shown, theoretically, that under the appropriate selective regimes, variability within family lines in inbreeding populations was possible (Wright 1921, Hayman and Mather 1953), and the morphological work of Jain and Allard (1960) demonstrated that such variability does exist in the inbreeding barley species. Later, quantitative measurements by electrophoretic techniques conclusively showed variability within family lines in a number of inbreeding plants (Allard and Kahler 1971).

For this report the genetic variability present in several local cultivars of an inbreeding species, *Hordeum distichum* (barley), and an outbreeder, *Secale cereale* (rye), was measured by electrophoretic techniques. Using the two measurements of percentage heterozygosity and a genetic polymorphic index, these two are compared in relation to their different breeding systems.

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If inbreeding species have variable loci, then evolutionary forces other than the breeding system must be acting upon them. This report also presents evidence that supports selection in the form of heterozygote advantage at the variable loci as the major force responsible for the development and maintenance of the genetic variability observed.

MATERIALS AND METHODS

Barley cultivars Research (officially known as Rupe), Lara, Zephyr and Carlsberg, and rye cultivars CRD and Rahu were investigated for this report.

All seeds were treated for 48 h at 4°C to break post-ripening dormancy, and were then germinated in a growth room at 25°C with an 8 h day for a further 48 h. The whole seedlings were crushed in 0.15 ml of gel buffer using a glass rod. This was then centrifuged, and 50 µl of the supernatant used to load the starch gels. At least 42 individuals of each cultivar were tested. The starch gels were prepared according to the method of Sargent (1969), and set in trays 80 mm x 225 mm x 6 mm with removable sides and a slot-former 60 mm from one end. The buffers used were those described by Kahler and Allard (1970). All electrophoretic separations were carried out at 4°C to prevent overheating, and were run for about 18 h. When the run was completed, the gel was cut horizontally into two 3 mm thick slices, one of which was assayed for esterase (E) and the other for acid phosphatase (AP) enzyme activity. The staining procedures followed were essentially those described by Smith (1968), and employ diazon coupling to detect the α-naphthyl released by enzyme action upon α-naphthyl acetate or α-naphthyl acid phosphate. For the esterase enzyme, the gel slice was first soaked for 1 h in 0.5 M boric acid to lower the pH of the gel to about 6.0. When the gel slices were removed from the stain solutions they were washed with distilled water and the band distances relative to the broate front measured.

RESULTS

Each band showing on the gel slices represents an allele product, so that the distribution of bands is determined by the genotype for each enzyme. Table 1, which presents data on 6 bands for 42 seedlings, is an example of this scoring procedure. Each band is represented by an R_M value (distance migrated relative to the movement of the borate front). The allelic combinations were determined by inspection directly from the band scoring results. If a particular band was present in all individuals then the corresponding locus was considered to be monomorphic, whereas, if a band was present in some individuals but absent in others then the locus was considered to be polymorphic.

From the band scoring results (Table 1), genotypic frequencies were determined for the cultivars of both species. This heterozygosity information is summarised in Table 2, which gives the mean heterozygosity of barley and rye as statistically significant ($P < 1\%$) by a modified t-test (Sokal and Rohlf 1969). Of the barley cultivars, Carlsberg shows the lowest degree of polymorphism;

TABLE 1. ACID PHOSPHATASE BANDING PATTERN IN BARLEY CULTIVAR ZEPHYR.

Run numbers	R_F values	Samples 1	2	3	4	5	6	7
	0.96	+	+	+	+	+	+	+
	0.94	+	+	+	+	+	+	+
1	0.75	+	-	-	+	+	-	+
	0.53	-	-	+	+	+	-	-
	0.45	-	+	-	-	+	+	-
	0.26	+	+	+	-	-	+	+
	0.96	+	+	+	+	+	+	+
	0.94	+	+	+	+	+	+	+
2	0.75	+	+	-	+	+	+	-
	0.53	-	+	-	-	-	+	+
	0.45	-	-	+	-	-	-	+
	0.26	+	-	+	+	+	-	+
	0.96	+	+	+	+	+	+	+
	0.94	+	+	+	+	+	+	+
3	0.75	+	+	-	+	+	+	-
	0.53	-	+	-	-	-	-	+
	0.45	-	-	+	-	-	-	+
	0.26	+	+	+	+	+	+	+
	0.97	+	+	+	+	+	+	+
	0.94	+	+	+	+	+	+	+
4	0.75	+	-	+	+	+	+	+
	0.53	-	-	-	+	+	-	-
	0.45	-	+	-	-	-	-	-
	0.26	+	+	+	-	-	+	+
	0.96	+	+	+	+	+	+	+
	0.94	+	+	+	+	+	+	+
5	0.75	+	+	+	+	+	+	-
	0.53	+	-	+	-	+	-	-
	0.45	-	-	-	-	+	-	+
	0.26	-	+	-	+	-	+	+
	0.96	+	+	+	+	+	+	+
	0.94	+	+	+	+	+	+	+
6	0.75	+	-	-	+	+	-	+
	0.53	+	-	-	-	-	-	+
	0.45	+	+	+	-	-	+	+
	0.26	+	+	+	+	+	+	-

TABLE 2. AVERAGE HETEROZYGOSITY OF THE BARLEY AND RYE CULTIVARS.

	Barley				Rye	
	Research	Lara	Zephyr	Carlsberg	CRD	Rahu
Varieties Percent heterozygosity	5.04	4.59	5.09	2.37	28.06	27.00
Species Percent		4.27			27.53	

its heterozygosity is close to half that shown by the three others. The second comparison of the polymorphism found within and between the barley and rye species was made using a polymorphic index,

$$PI = \sum_{i=1}^{N} P_i\ (1-P_i)/N$$

in which p_i is the frequency of the ith band and N is the number of bands (Marshall and Allard 1970), see Table 3. The difference between the mean p_i values for barley and rye was significant ($P < 2\%$) by the modified t-test. The barley cultivar Carlsberg again showed the lowest level of polymorphism within the barleys, at least in these two enzyme systems.

In a quantitative comparison of these results for barley and rye, the forces likely to govern genetic variability, in particular the breeding systems of the plants and any selective pressures present, must be taken into consideration. For an inbreeding population, initially non-inbred and with neutral alleles at each locus, the amount of heterozygosity present after n generations is directly proportional to the Inbreeding Coefficient F_e.

$$F_e^n = s/1+t\ (1 - (s/2)^n)$$

where there is a proportion t of random outcrossing and a proportion s = (1-t) of selfing (Allard *et al.* 1972). The four barley cultivars used, with the exception of cultivar Lara, have been established for at least 15 years, and therefore inbreeding equilibrium was assumed to have been reached. The determination of exact t values for the four cultivars was beyond the scope of this investigation, so a value of t = 0.57% (from Allard *et al.* 1972) was used. If other factors in addition to the breeding system, such as selection, mutation, migration and random genetic drift are involved, then the combined effect of them all is designated the Fixation Index $\hat{F}$. This is defined by Weir (1970) as,

$$\hat{F}_{ij} = 1 - f_{ij}/p_i p_j$$

where $2f_{ij}$ is the observed heterozygote frequency and p_i and p_j are the allele frequencies for the ith and jth alleles. Table 4 shows the equilibrium Inbreeding Coefficients and the Fixation Indices for the polymorphic loci of the barley cultivars. A consistent feature in Table 4 is that the $\hat{F}$ values are lower than the F_e values; these indicate an excess of heterozygotes over those expected on the basis of the breeding system alone. The genetic variability measured in this barley species must therefore have some force or forces acting to maintain it above the level predicted purely by its inbreeding system.

The importance of selection upon the heterozygosity found was clearly demonstrated when estimates of selective values for the homozygotes and heterozygotes were made. These estimates were calculated using the maximum likelihood estimator of Workman and Jain (1966: Model 1). These selective values are given in Table 5 and show that the heterozygotes have fitness approximately twice those of the homozygotes. This result makes it seem likely that selection and the breeding system are the major forces responsible for the observed heterozygote frequencies in these barley cultivars.

TABLE 3. POLYMORPHIC INDICES OF THE BARLEY AND RYE CULTIVARS.

Enzyme system	Barley				Rye	
	Research	Lara	Zephyr	Carlsberg	CRD	Rahu
Esterase	0.151	0.148	0.157	0.074	0.211	0.226
Acid phosphatase	0.131	0.123	0.141	0.139	0.217	0.208
Variety mean	0.141	0.135	0.149	0.106	0.214	0.217
Species mean		0.133			0.216	

TABLE 4. INBREEDING COEFFICIENTS AND FIXATION INDICES FOR THE BARLEY CULTIVARS AT INBREEDING EQUILIBRIUM.

Locus	Inbreeding Coefficient	Fixation Indices			
		Research	Lara	Zephyr	Carlsberg
E_D	0.988	0.782	0.833	0.818	0.813
E_C	0.988			0.777	
E_B	0.988	0.672	0.789		
AP_C	0.988			0.832	
AP_B	0.988	0.752	0.803		0.786
AP_A	0.988	0.875	0.739	0.717	0.845

TABLE 5. ESTIMATED SELECTIVE VALUES FOR HOMOZYGOTES AT EACH LOCUS RELATIVE TO THE SELECTIVE VALUE OF THE HETEROZYGOTES TAKEN AS UNITY.

Locus	Genotype	Research	Lara	Zephyr	Carlsberg
E_D	0.86/0.86	0.378	0.482	0.468	0.467
	0.54/0.54	0.358	0.529		
	0.36/0.36			0.521	0.521
E_C	0.68/0.68			0.487	
	0.60/0.60			0.509	
E_B	0.46/0.46	0.509	0.495		
	0.15/0.15	0.441	0.506		
AP_C	0.75/0.75			0.535	
	0.45/0.45			0.526	
AP_B	0.62/0.62	0.506	0.510		0.534
	0.13/0.13	0.471	0.487		0.539
AP_A	0.53/0.53	0.547	0.510	0.478	0.509
	0.39/0.39		0.464		
	0.26/0.26	0.532		0.555	0.512

DISCUSSION

For large, sexually-reproducing plant populations, about 12% heterozygosity seems to be an average value (Allard and Kahler 1971), although heterozygosity as high as 35% has been reported (Levin 1975). The estimated 27% heterozygosity for the rye species in this investigation is therefore very high. However, this figure may reflect large local variation in genetic diversity and does have a large standard error because of the low sample size used. The observed mean 4% heterozygosity of the barleys agrees well with the 2% to 4% heterozygosity quoted by Allard and Kahler (1971) as being typical of highly inbreeding plants. This degree of polymorphism is in the higher part of the range shown by the barley species (see Allard *et al.* 1970), so it seems these barley cultivars are examples of the more polymorphic types existing.

The statistically significant difference between barley and rye in both the percentage heterozygosity and the polymorphic index indicates a considerable difference in the genetic variability between them. This result suggests that the amount of genetic variability measured in plant species may be correlated with their breeding systems. However, genetical structure can differ between similar species, and Levin (1975), from a survey of 19 different species, concluded that such a correlation does not exist.

The classical inbreeding concepts (see Stebbins 1950, 1957) predicted that heavy inbreeding leads to homozygosity and so to genetic uniformity. The variability in the inbreeding barley species found was significantly lower than in the outbreeding rye species, but the 4% heterozygosity in the barley refutes the classical assumption of almost total homozygosity. That appreciable amounts of heterozygosity can exist in at least some inbreeding species has been the major conclusion of electrophoretic investigations into such plants, and so a reassessment of the classical concepts is warranted. Perhaps the suggestion of Jain *et al.* (1970) that a wide spectrum of genetical responses is possible under inbreeding, with the classical concepts of homozygosity and Allard's variability measurements just representing opposite extremes, is closest to the true situation.

Any model of inbreeding which allows large amounts of genetic variability, particularly heterozygosity, must be able to explain its maintenance. A number of theoretical papers, reviewed by Allard *et al.* (1968), showed that, with the appropriate selective pressures, variability can be maintained indefinitely under conditions of inbreeding. In these investigations, Wright's F statistics have been the most successful indicator of the forces present in inbreeding populations. In agreement with those of Allard *et al.* (1972), the results of this investigation show a consistent excess of the Inbreeding Coefficient over the Fixation Index, indicating that the breeding system alone will not explain the heterozygosity. Of the other possible forces mutation can be discounted as a significant factor (Clegg and Allard 1973). Migration can be eliminated as the populations used are commercial seed cultivars and, because the grain stocks sampled are very large, sampling errors contributing to genetic drift would be minimal. Thus the breeding system, the importance of which has already been discussed, and selection are left as the two major forces

determining the genetic variability seen in barley. The measurement of selective values which showed that the heterozygotes are favoured just over twice as much as the homozygotes confirms the above conclusion.

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